ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 2, 173-180
www.aaem.pl

Mouthwash and Oral Cancer Risk – Quantitative Meta-analysis of Epidemiologic Studies

Sara Gandini[1,2], Eva Negri[2,3], Paolo Boffetta[2], Carlo La Vecchia[2,3], Peter Boyle[2]

[1] *European Institute of Oncology, Milan, Italy*
[2] *International Prevention Research Institute, Lyon, France*
[3] *Istituto di Ricerche Farmacologiche 'Mario Negri', Milano, Italy*

Gandini S, Negri E, Boffetta P, La Vecchia C, Boyle P. Mouthwash and Oral Cancer Risk – Quantitative Meta-analysis of Epidemiologic Studies. Ann Agric Environ Med. 2012; 19(2): 173-180.

Abstract

Background: Use of mouthwash and an increased risk of oral cancer has been a source of controversy for decades. A meta-analysis of epidemiological studies of mouthwash and oral cancer and, specifically, mouthwash containing >25% alcohol, was undertaken.

Methods: Summary estimates were obtained with maximum likelihood estimates from random effects models. Sensitivity analyses were conducted to evaluate the influence of various inclusion.

Results: Eighteen studies were included in the meta-analysis. There was no statistically significant associations found between regular use of mouthwash and risk of oral cancer (RR=1.13; 95% CI (0.95-1.35)). There was no significant trend in risk of oral cancer associated with increased daily usage of mouthwash (p=0.11). There was no association between reported use of mouthwash specifically containing alcohol and risk of oral cancer (RR=1.16; 95% CI (0.44, 3.08)).

Conclusions: This quantitative analysis of mouthwash use and oral malignancy revealed no statistically significant associations between mouthwash use and risk of oral cancer, nor any significant trend in risk with increasing daily use; and no association between use of mouthwash containing alcohol and oral cancer risk.

Key words

Oral cancer, alcohol consumption, tobacco smoking, Mouthwash

INTRODUCTION

There are an estimated half-a-million of cases of cancer of the oral cavity and pharynx occurring annually, and a quarter-of-a-million deaths [1]. The higher rates (incidence and mortality) occur in Central Europe and France [2, 3] and on the Indian sub-continent [4].

The majority of oral cancers are squamous-cell carcinoma (SCC), and the main risk factors for these cancers are tobacco and alcohol use. Tobacco smoking is the most important risk factor for head and neck cancer, and the risk is higher for heavy smokers, long-term smokers and smokers of black tobacco or high-tar cigarettes. Cigar and pipe smoking also pose a risk, while stopping smoking is followed by a decrease in risk [5]. Smoking of bidis (small cigarettes common in parts of Asia) also carries a substantial risk of oral cancer [6].

Consumption of alcoholic beverages also increases the risk of oral cancer, and other cancers of the head and neck. Relative to abstainers and very light drinkers, the risk in heavy drinkers is in the order of tenfold. Although the effect of alcohol and tobacco may vary slightly according to the different sub-sites, the combined effect of both exposures accounts for the majority of all head and neck cancers that occur globally.

Address for correspondence: Peter Boyle, International Prevention Research Institute, 95 cours Lafayette, 69006 Lyon, France
Email: peter.boyle@i-pri.org

Received: 28 March 2012; accepted: 01 June 2012

A recent pooled analysis from the INHANCE consortium based on over 10,000 cases and 15,000 controls, shows that approximately 70% of such cancers can be explained by these two exposures, ranging from 65% for oral cavity cancer (51% for women and 65% for men) to 86% for cancer of the larynx (79% for women and 86% for men). The proportion of those cancers caused by alcohol and tobacco was reduced with decreasing age, being just 32% for cancers diagnosed prior to the age of 45. Strong interaction between the two exposures are also apparent.

In addition to the dominant roles of tobacco smoking and alcohol drinking in the causation of oral cancer, other established risk factors specifically for oral cavity cancer are betel quid and areca nut in India and Taiwan [7, 8], and poor oral health [9]. Chronic infection with Human Papillomavirus (HPV) is emerging as an important risk factor, particularly for cancer of the tongue and oro-pharyngeal cancer [10].

In Europe, the distribution pattern of oral cancers follows that of alcohol consumption. While the incidence is decreasing in many countries, it is still on the rise in Central Europe. Mortality is also declining in many countries, but is still very high in Central Europe and still increasing among younger birth cohorts.

Alcohol drinking, ethanol and acetaldehyde associated with alcohol drinking, have been identified as human carcinogens [11]. Acetaldehyde from drinking alcohol was considered a human carcinogen, based on studies from Japan regarding risk of oesophageal cancer.

In recent studies, it has been shown that the relative risk of oral cancer increases with the average daily amount consumption of alcohol. The total ethanol content of alcohol drunk has been consistently demonstrated to be the main factor in determining cancer risk. Alcohol also increases the permeability of the oral mucosa to Tobacco Specific Nitrosamines (TSNs), and potentially other carcinogens. Short-term exposure to alcohol increases the permeability of the human oral mucosa.

Ethanol is contained in a number of ready-to-use mouthwashes in a concentration typically between 5 - 27% volume. There are two main questions to be resolved:

1) whether there is a threshold for alcohol consumption in increasing oral cancer risk;
2) is there any risk associated with rinsing the mouth with an alcohol-containing mouthwash which is not consumed?

The potential association between use of mouthwash and an increased risk of oral cancer has been a source of controversy for decades. In recent times, attention has focused on a role for those mouthwashes containing alcohol. There have been reports in the scientific literature, spread over the past thirty years, investigating the potential association between mouthwash use and its impact on the risk of oral cancer. Epidemiological studies have been relatively few and frequently contradictory.

Mouthwashes contain a variety of active and inactive ingredients. The ingredients of a mouthwash include antibacterial agents, at least 50% water, stabilizers for non-water soluble ingredients, substances to improve palatability and stability, and preservatives to increase shelf-life. Ethanol is used in some mouthwash formulas as a solubiliser, stabilizer, preservative, sensory cue with a distinctive taste, and as an anti-plaque efficacy enhancer (adjuvant effect). Ethanol at 18-27% concentration enhances the effect of essential oils (high penetration achieved in 30 seconds).

In order to clarify the issue of mouthwash use and oral cancer risk, a comprehensive literature review and formal meta-analysis was carried out on mouthwash use and oral cancer, oropharyngeal cancer and oropharyngeal and laryngeal cancers.

MATERIALS AND METHODS

A systematic literature search and quantitative analysis was planned, conducted and is reported following the MOOSE guidelines regarding meta-analysis of observational studies [12].

Definition of Exposures and Outcome. The definition used for the exposure variable is 'regular use of mouthwash' which was classified as regular use on average 'once or twice a day'. When risk estimates for more than one definition were presented, definitions such as 'daily use' and 'ever use' were preferred to 'higher doses' (e.g. more than twice a day). Whenever possible, the estimates for mouthwash with a specified content of alcohol>25% were chosen.

The outcome variable was 'Oral Cancer' but estimates for oral and pharyngeal cancers together were also included, relying on the definition as published in each report.

Data Sources and Search Strategy. Published reports were obtained from the following databases using validated search strategies: Ovid MEDLINE database; ISI Web of Science Science Citation Index Expanded (SCI Expanded); and PUBMED (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). Other sources were found in the reference lists of the retrieved articles and preceding reviews on the topic.

The following search terms (both as MeSH terms and as keywords) were used to identify potentially relevant studies in the three databases mentioned above: oral, oral-pharyngeal, oral-pharyngeal-laryngeal cancer, leukoplakia or oral epithelial dysplasia and mouthwash, oral rinse or Lysterine. The search was limited to human studies but no language or time restrictions were applied.

Selection of Articles. All searches were made independently by two abstractors (S. Gandini and E. Negri); in case of disagreement or uncertainty, a third reviewer (C. La Vecchia) was consulted.

Usual inclusion criteria were used for the selection of all relevant articles (i.e., case-control, cohort, or cross-sectional studies) published as an original article. These criteria included that studies should have sufficient information to allow adequate estimation of the relative risk (RR) and 95% confidence intervals (95% CI): i.e., the authors should report either adjusted odds ratios or RRs or crude data and SEs, variance, CIs, or P values of the significance of the estimates; and that the studies should be independent to avoid giving double weight to some estimates.

Extraction and Classification of the Data. For each study, the following data were retrieved:

1. *Study:* publication year, study design, study location, mean age of study population, gender;
2. *Exposure:* definition of the types of use of mouthwash and time of ascertainment of mouthwash (how long before cancer diagnosis?);
3. *Cases:* number and source of cases, accrual period, histological confirmation, type of registration: incident vs. prevalent cases;
4. *Controls:* number and source of controls, matching design, inclusion/exclusion of specific types of diseases/cancers;
5. *Statistics:* statistical methods used and adjustment for confounding variables (e.g. smoking and/or alcohol consumption), restriction of analysis on specific subgroup (smokers, non-smokers, non-drinkers …).

Fully adjusted RRs, when available, were retrieved for each dose of mouthwash use, for all the population under study and for smokers, non-smokers/non-drinkers, by cancer sub-sites, and by gender.

Statistical Methods. The various estimates of RR and their CIs were transformed into log RR and the corresponding variance was calculated using the formula proposed by Greenland [13]. When estimates were not given, they were calculated from tabular data and using Woolf's formula to evaluate the SE of the log odds ratio [13].

The homogeneity of the effect across studies was assessed by using the large sample test based on the chi-square statistic. Because this test has limited power, statistically significant heterogeneity was considered to be at the p=0.10 level of association. Heterogeneity across studies was also evaluated by I^2, which represents the percentage of total variation across studies, attributable to heterogeneity rather than to chance [14].

Random effects models were used, including the two sources of variation (within and between studies), to take into account correlation within the study when more than one estimate was extracted from a single study. Summary estimates were obtained with maximum likelihood estimates from random effects models (REF Proc Mixed in SAS software [version 8.02; SAS Institute, Cary, NC] [15].

Sub-group analyses and meta-regression were carried out to investigate between-study heterogeneity, and to evaluate the effect on the summary estimates of study features, types of population, types of mouthwash definitions, and use. Sensitivity analyses were carried out to evaluate the influence of various inclusions/exclusion criteria and specific studies.

For dose-response estimates, RRs, 95% CIs and number of cases and controls were retrieved by each category of exposure. Within each study, we used a linear model to estimate the RRs associated with an increase in mouthwash use of one time/d.

Each category of mouthwash use was assigned the value corresponding to the mid-point of the range. Summary RRs were obtained by pooling the study-specific estimates by the random-effects models proposed by Greenland and Longnecker [16], which adjust the estimates for within-study co-variance and accounts for the correlation between estimates. Estimates for 2 and 3 times a day were estimated from the linear dose-response model [16].

The impact of whether publication bias might affect the validity of the estimates was investigated using a funnel-plot–based approach: the regression of ln(RR) on the sample size, weighted by the inverse of the variance [17].

RESULTS

Literature search and data extraction. Through the literature searches, 18 full-text articles were found that were considered for inclusion in the meta-analysis. Two studies were excluded because they were not independent [18, 19] (Fig. 1).

Features of the 16 studies included in the main analysis and in the sensitivity analyses are presented in Table 1, the estimates reported by the authors are presented in Table 2.

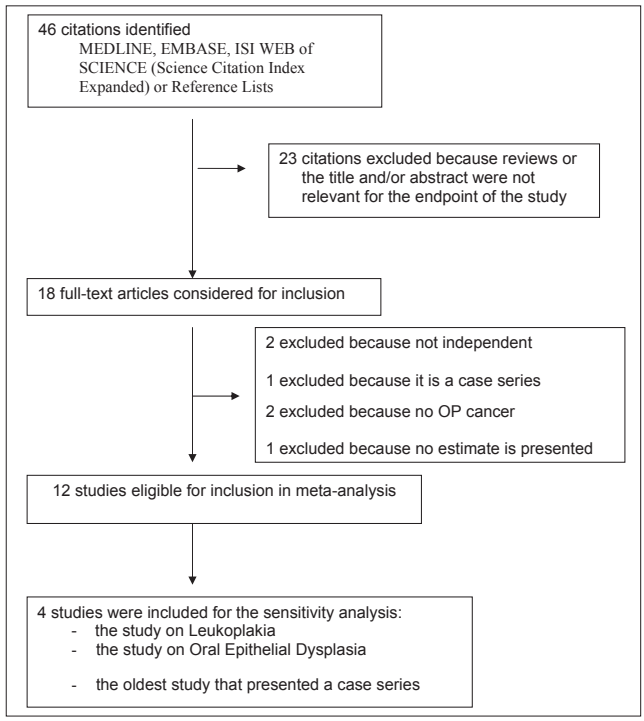

Figure 1. Flow chart of selection of studies for inclusion in meta-analysis.

Table 1. Study characteristics of article evaluated in meta-analysis

FA	PY	Study period	Gender	Design	Country	Cancer type	Info Source	n. cases	n. controls	% regular use cases	% regular use contr.
Blot	1983	1975-78	W	CC	USA	OP	Hospital	206	352	44%	42%
Wynder	1983	1977-80	M+W	CC	USA	OP	Hospital	571	568	47%	56%
Mashberg	1985	1981-83	M+W	CC	USA	OP	Hospital	95	913	43%	48%
Young	1986	NA	M+W	CC	USA	O	Hospital	202	306	NA	NA
Kabat	1989	1983-87	W	CC	USA	OP	Hospital	125	107	29%	33%
Winn	1991	1984-85	M+W	CC	USA	OP	Populat.	866	1,249	54%	44%
Talamini[1]	2000	1996-99	M+W	CC	Italy	O	Hospital	121	137	9%	9%
Winn	2001	1992-95	M+W	CC	USA	OP	Populat.	328	496	33%	38%
D'Souza	2007	2000-05	M+W	CC	USA	OP	Hospital	100	200	40%	36%
Guha[2]	2007	1998-03	M+W	2CC	Mixed	OP	Hospital	918	2,752	5%	3%
Divaris	2010	2002-06	M+W	CC	USA	O	Populat.	692	1,361	NA	NA
Macfarlane[3]	2010	NA	M+W	CC	Europe	OP	Populat.	260	340	11%	10%
Included only in the sensitivity analysis											
Weaver[4]	1979	NA	M+W	-	USA	O	Hospital	11	50	91%	80%
Marshall	1992	1975-83	M+W	CC	USA	OP	Hospital	290	290	NA	NA
Morse	1997	1990-93	M+W	CC	USA	OED	Hospital	127	127	41%	47%
Mascarenhas	2002	1997-98	M+W	CC	USA	Leukoplakia	Hospital	58	58	10%	10%

O: Oral cancer. OP: Oral-Pharyngeal cancer. OED: Oral epithelial dysplasia. M: men; W: women. CC: case-control study.
1. Cases evaluated included oral cavity and pharynx, controls used for pharynx: 1,378 and 1,225 for oral cavity;
2. Frequency of use in cases, on average, 2 or more a day; in controls, 'at least occasionally';
3. 5% of cases are oesophagus cancers.
4. Frequency of regular use: > 2 a week; cancer; regular use: 1 or more times a day.

Table 2a. Risk estimates of studies evaluated in meta-analysis

FA	Gender	cancer	n cases	n controls	% regular use Cases	% regular use Controls	*OR*	Smk or alc. Adj.	Exposure Definition	Smokers or alcohol drinkers	% of alcohol	Controls
Weaver, 1979[3]	3	O	11	50	91%	80%	2.5 (0.29, 21.88)	0	2+ daily for 20 years	Neither*	majority 27% alcohol	Male surgery pts.
Blot, 1983	2	OP	206	352	44%	42%	1.15 (0.8, 1.7)	1	Ever use on a regular basis			
	2	OP	31	138			1.94 (0.8, 4.7)	1		No Tobacco		
Wynder, 1983	2	OP	157	157	57%	46%	1.54 (0.82, 2.89)	0	< 1 a day			
	1	OP	414	411			0.79 (0.55, 1.15)	0	<1 a day			
	2	OP					2.79 (1.67, 4.66)	0	1 or more times a day			
	1	OP					1.13 (0.83, 1.54)	0	1 or more times a day			
	2	OP	36	88			3.63 (1.48, 8.92)	0	Daily use	Neither*		
	1	OP	9	105			0.23 (0.03, 1.79)	0	Daily use	Neither*		
Mashberg, 1985[2]	3	OP	95	913	43%	48%	0.94 (0.61, 1.47)	1	4 times weekly			Non tobacco related cancers
	3	OP	10	396			2.01 (0.52, 7.66)	0		No Tobacco		
	3	OP	28	508			0.83 (0.39, 1.77)	0		No alcohol		
	3	OP	41	438			0.57 (0.29, 1.13)	0			≥25%	
Young, 1986	2	O	52	155			0.52 (0.25, 1.1)	0	MW users			Cancer pts (including larynx)
	1	O	150	468			1.02 (0.67, 1.56)	0				
	2	Oroph. +Hyp.	27	155			0.55 (0.22, 1.4)	0				
	1	Oroph. +Hyp.	88	468			0.96 (0.52, 1.5)	0				
	2	O					0.41 (0.12, 1.43)	0		No Tobacco		
	1	O					2.63 (0.5, 13.73)	0		No Tobacco		

Table 2b. Risk estimates of the studies evaluated in the meta-analysis

FA	Gender	cancer	n cases	n controls	% regular use Cases	% regular use Controls	*OR*	Adj.	Exposure Definition	Smokers or alcohol drinkers	% of alcohol	Controls
Kabat, 1989	2	OP	124	107	29%	33%	0.74 (0.40, 1.49)	1	Regular use for at least 1 year, 10 years before diagnosis			Cancer pts (including larynx)
	2	OP					0.94 (0.39, 2.28)	1	Occasional use, 10 ys before diagnosis			
	2	OP					1.38 (0.42, 4.55)	1	Daily	Neither*		
	2	OP					0.74 (0.4, 1.4)	1	Regular use for at least 1 year, 10 years ago			
Winn, 1991	2	OP	293	428	58%	45%	1.9 (1.1, 3.3)	1	≥1 a week for 6 months		≥25%	
	1	OP	573	821	49%	44%	1.6 (1.1, 2.3)	1			≥25%	
	2	OP					1.6 (1.1, 2.3)	1				
	1	OP					1.4 (1, 1.8)	1				
	2	OP	30	108			1.1 (0.5, 2.6)	1		Neither*		
	1	OP	11	63			1.3 (0.3, 4.6)	1		Neither*		
Marshall, 1992	3	OP	290	290			Significant risk elevation	NA	Recent users			

Table 2b (Continuation). Risk estimates of the studies evaluated in the meta-analysis

FA	Gender	cancer	n cases	n controls	% regular use Cases	% regular use Controls	*OR*	Adj.	Exposure Definition	Smokers or alcohol drinkers	% of alcohol	Controls
	3	OED	127	127	41%	47%	0.8 (0.4, 1.5)	1	1+ uses/ week for 6 months			
Morse, 1997	2	OED	51	51			0.5 (0.2, 1.4)	1				
	1	OED	76	76			1.3 (0.5, 3.4)	1				
	3	OED					1 (0.3, 3.4)	1		No Tobacco		
	3	OED					0.5 (0.2, 1.4)	1			≥25%	

Table 2c. Risk estimates of the studies evaluated in the meta-analysis

FA	Gender	cancer	n cases	n controls	% regular use Cases	% regular use Controls	*OR*	Adj.	Exposure Definition	Smokers or alcohol drinkers	% of alcohol	Controls
Talamini, 2000[4]	3	OP	121	137	9%	9%	1.5 (0.5, 3.8)	1	1-2 times a week			
							1.2 (0.4, 3.5)	1	>2 a week			
Winn, 2001	3	OP	328	496	36%	41%	1 (0.7, 1.4)	1	1+ a week for >6 moths, 1 y. ago		≥25%	
	2	OP					2.1 (0.9, 5)	1			≥25%	
	1	OP					0.8 (0.5, 1.2)	1			≥25%	
	3	OP					1.1 (0.7, 1.8)	1	<2 a day		≥25%	
	2	OP					2.9 (1.0, 8.5)	1	<2 a day		≥25%	
	1	OP					0.8 (0.4, 1.4)	1	<2 a day		≥25%	
	3	OP					0.9 (0.5, 1.4)	1	≥2 a day		≥25%	
	2	OP					1.5 (0.5, 4.5)	1	≥2 a day		≥25%	
	1	OP					0.7 (0.4, 1.3)	1	≥2 a day		≥25%	
	3	O					1.1 (0.7, 1.8)	1				
	3	OP					2.8 (0.8, 9.9)	1		Neither*		
Mascarenhas, 2002	3	Leukoplakia	58	58	10%	10%	2.3 (0.4, 12)					
		Viadent rinse										
D'Souza, 2007	3	OP	100	200	40%	36%	1.3 (0.8, 2.1)	1	1-2 times a day			
	3	OP					3.8 (0.9, 16.5)	1	3-4 times a day			
Guha, 2007[1]	3	O	316	1225	5%	3%	1.13 (0.68, 1.85)	1	<1 a day			
	3	O					1.57 (0.8, 3.1)	1	1 a day			
	3	O					5.86 (2.91, 11.77)	1	≥2 a day			
	3	OP	81	413			1.54 (0.71, 3.37)	1	<1 a day	No Tobacco		
	3	OP					1.89 (0.45, 7.84)	1	1 a day	No Tobacco		
	3	OP					2.71 (0.74, 9.97)	1	≥2 a day	No Tobacco		
	3	OP	137	401			0.56 (0.21, 1.50)	1	<1 a day	Never drinkers		
	3	OP					4.27 (1.14, 16)	1	1 a day	never drinkers		
	3	OP					4.96 (1.85, 13.31)	1	≥2 a day	never drinkers		

Table 2d. Risk estimates of studies evaluated in meta-analysis.

FA	Gender	cancer	n cases	n controls	% regular use Cases	% regular use Controls	*OR*	Adj.	Exposure Definition	Smokers or alcohol drinkers	% of alcohol	Controls
Divaris, 2010	3	O	692	1361			0.97 (0.78, 1.22)	1	Regular use			
	3	OPL					0.95 (0.78, 1.15)	1	Regular use			
	3	OPL					0.96 (0.44, 2.12)	1		Neither*		
Macfarlane, 2010[5]	3	OPL	260	340	11%	10%	1.02 (0.66, 1.60)	1	< once a day			
	3	OPL					1.22 (0.65, 2.30)	1	Once a day			
	3	OPL					1.70 (0.73, 3.95)	1	2+ times a day			

1. No. of oral cancer cases with information on mouthwash use; % of regular use (once a day) on OP;
2. % refers to 'users', frequency of use per day not known;
3. frequency of use of cases, on average, 2 or more a day; in controls: 'at least occasionally';
4. No. of cases and controls with information on mouthwash use;
5. 5% of cases concern the oesophagus.

Twelve studies published between 1983 - 2010 were available for the main analysis (Tab. 1). All of them were case-control studies, 4 were population based [20, 21, 22, 23], one was conducted in Italy [24], one was a mixture (Latin American and Europe) [25], and one a mixture of European countries [23]. All the others were from United States [20, 26, 27, 28, 29, 30]. Two publications presented data on women [26, 30], 3 were on oral cancer [22, 24, 28], and all the others on oral and pharyngeal cancers. One study [23] included also 5% of oesophagus cancers among cases.

For one study [30], the estimate that refers to use 10 years before diagnosis was chosen instead of the most recent estimate because of the time-lag in exposure to cancer, and also because clinical manifestations of early oral cancer could modify the subject's use of an agent, such as a mouthwash (e.g. it might be used to treat symptoms).

Statistical analysis. No significant association was found between mouthwash use and oral cancer: SRR=1.13 (95%CI: 0.95; 1.35). (Forest plot, Fig. 2).

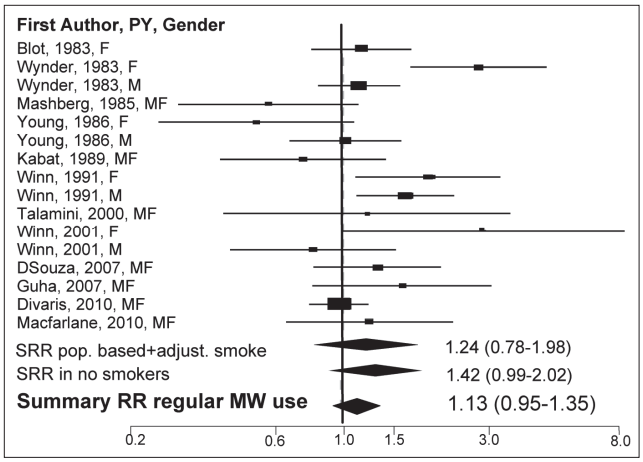

Figure 2. Forest plot for regular mouthwash use and oral cancer.

Nine studies were available for the dose-response analysis [20, 21, 23, 24, 25, 26, 27, 30, 31]. RRs for 3 'doses' were abstracted: 2 or more times a day, once a day, and no exposure to mouthwash. The summary relative risks estimates for 1-3 times a day of mouthwash showed no statistically significant increase risk for oral cancer, compared to no exposure: 1.19 (95%CI: 0.95, 1.5), 1.42 (95%CI: 0.91, 2.24) and 1.7 (95%CI: 0.86, 3.35), respectively, with I^2=76% and Chi-square p<0.001. (Forest plot, Fig. 3).

Sub-group and sensitivity analyses were carried out, including risk estimates for: oral cancer only (excluding oral-pharyngeal cancers); only non-smokers (and non-drinkers when possible); only smokers; mouthwash with specified 25% of alcohol content; high dose of mouthwash use (2+ times a day were chosen when possible); population based studies publishing estimates adjusted for smoking and preferably alcohol consumption; and all possible studies, including the 4 studies excluded from the main analysis [32, 33, 34, 35).

Weaver [32] was the first to publish a study on mouthwash and oral cancer, and involved a case series of 11 women with oral cancer (10 of whom were heavy users of mouthwash), and compared them with 50 men. For this study, an estimate of cancer risk was obtained from the percentages of mouthwash use presented in the text. Mascarenhas et al. [33] evaluated the effect of mouthwash use on leukoplakia. Morse et al.

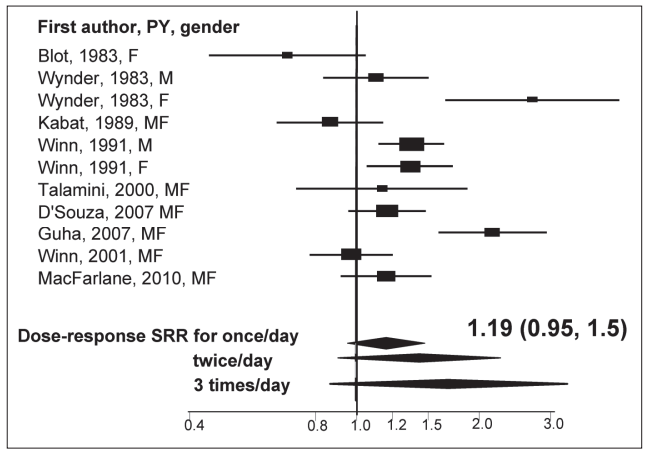

Figure 3. Forest plot from dose-response models on number of times per day of mouthwash use and oral cancer.

[34] evaluated the effect of mouthwash use on oral epithelial dysplasia. Although Marshall et al. [35] did not publish an estimate for mouthwash use and oral cancer, their Results described a significant effect ofr regular use. In order to be conservative, the estimate of this study was imputed considering the highest estimate published by the other authors.

Summary risk estimates for subgroup analyses and sensitivity analyses are presented in Table 3. None of the factors evaluated through meta-regression significantly explained between study heterogeneity (Publication year: p=0.66; gender p=0.36) and no evidence of publication bias was found (p=0.31).

Table 3. Summary relative risk estimates for mouthwash use

n studies	RR 95%CI	I^2%	P-χ^2	Definitions
Main analysis on Oral-Pharyngeal cancer				
12	1.13 (0.95; 1.35)	58	0.002	Ever use
9	1.19 (0.95, 1.5)	76	<0.001	Once a day
Sensitivity analyses				
4	0.99 (0.75; 1.31)	19	0.30	Only oral cancer
10	1.42 (0.99; 2.02)	21	0.23	In no smokers
6	0.89 (0.74; 1.07)	97	<0.001	In smokers
3	1.16 (0.44; 3.08)	72	0.01	With alcohol content at 25%
12	1.31 (0.91; 1.88)	74	<0.001	OP cancer with high use
4	1.24 (0.78; 1.98)	94	<0.001	Pop based and adj. for smoking
16	1.19 (0.98; 1.44)	70	<0.001	Including all possible studies

DISCUSSION

The potential association between use of mouthwash and an increased risk of oral cancer has been a source of controversy for several decades, since the initial observations of Weaver et al. [32]. Evaluation of the available published epidemiological information in the 1990s concluded that there was no such association [36, 37, 38].

In recent times, attention has focused on a role for those mouthwashes containing alcohol on impacting the risk of oral cancer. This study set out to examine in a quantitative manner the potential effect of mouthwash use, and particularly use of mouthwash containing a high alcohol content, on the risk of oral cancer. All published studies were identified using a thorough literature review and examination of reference lists in published articles.

Standard criteria were used to determine which of the identified studies should be included in the analysis. Studies were required to have sufficient information to allow adequate estimation of the relative risk (RR) and 95% confidence intervals (95% CI). Following this strategy, 18 full-text articles suitable for inclusion in the main analyses and some in selected sensitivity analyses. This ensured that the maximum amount of information on the subject could be employed. However, the quality of many of the available studies from the epidemiological viewpoint is relatively poor, and mouthwash use has rarely been the principal hypothesis investigated in these studies.

There was no statistically significant association found between regular use of mouthwash and risk of oral cancer (RR=1.13; 95% CI (0.95-1.35)). There was no significant trend in the risk of oral cancer associated with increased daily usage of mouthwash (p=0.11). In sensitivity analyses, there was no association found when analysis was restricted to a number of factors, including oral cancer only, smokers, non-smokers and when all possible studies were included. There was no association between reported use of mouthwash specifically containing alcohol and risk of oral cancer (RR=1.0; 95% CI (0.39, 2.60)).

In studying the association of mouthwash and oral cancer it is important to bear in mind that there may be risk determinants, as well as effect modifiers and confounders involved. Mouthwash may act as a confounder for tobacco smoking (smokers may use mouthwash to cover the tobacco smell in their mouth), and mouthwash may effect tobacco smoking and alcohol drinking by acting as an effect modifier. There is very limited information available in the studies regarding why mouthwash is being used; it would therefore be very useful to have this information, particularly concerning those who volunteered that they were frequent daily users. Poor oral hygiene appears to be associated with increased risk of oral cancer, independent of any effect of tobacco and alcohol consumption, and more information is needed about the tendency and use of mouthwash among persons at increased risk of oral cancer due to poor oral hygiene.

The role of mouthwash use in the etiology of oral carcinogenesis must be viewed in the wider context of the biology of the mouth, the biology of oral carcinogenesis, and oral cancer epidemiology. Further evaluation of what has already been published would be valuable, in particular a re-analysis of existing studies, in order to properly control confounders, especially in older studies when statistical methods, such as logistic regression, were not widely available. Above all, there is a need to undertake studies in which more attention is given to the investigation of the effect of mouthwash use at different points throughout the life of subjects, with a focus on the reasons for using mouthwash and the particular types of mouthwash used.

The presented quantitative analysis of all published epidemiological studies of mouthwash use and oral malignancy revealed:

1) no statistically significant association between mouthwash use and risk of oral cancer, including no significant trend in risk with increasing daily use;
2) no association between use of mouthwash containing alcohol and oral cancer risk. However, it remains clear that more epidemiological studies are needed which will have a greater focus on certain aspects of mouthwash use and the development of oral cancer.

Acknowledgements

The International Prevention Research Institute was awarded an unrestricted research grant from J & J, manufacturers of one type of mouthwash, to study the effects of mouthwash on several aspects of dental health, including plaque, gingivitis and tooth decay. None of the authors received any honorarium in connection with this project.

REFERENCES

1. Boyle P and Levin B (eds). World Cancer Report 2008. IARC, Lyon, 2008.
2. Macfarlane GJ, Boyle P, Evstifeeva TV, Robertson C, Scully C. Rising Trends of Oral Cancer Mortality World-wide, The return of an old Public Health Problem. Cancer Causes and Control. 1994; 5: 259-265.
3. Boyle P, Smans M. Cancer Mortality Atlas of European Union, 1993-1997. IARC Press, IARC ,Lyon; 2008.
4. Curado MP, Edwards B, Shin HR, Storm H, Ferlay J, Heanue M, Boyle P. (eds). Cancer Incidence in Five Continents. IARC Scientific Publications 2007; IX(160).
5. International Agency for Research on Cancer Working Party. Eversal of Risk after quitting smoking. IARC Handbooks of Cancer Prevention, Tobacco Control, Volume 11, IARC, Lyon; 2006.
6. Sapkota A, Gajalakshmi V, Jetly DH, et al. Smokeless tobacco and increased risk of hypopharyngeal and laryngeal cancers: a multicentric case-control study from India. Int J Cancer. 2007; 121: 1793-1798.
7. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 85. Betel-quid and Areca-nut Chewing and Some Areca-nut derived Nitrosamines. IARC, Lyon; 2006.
8. Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V. WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol. 2009; 10(11): 1033-4.
9. Zheng T, Boyle P, Hu J, Duan P, Jiang D, Ma L, Shui L, Niu S, Scully C, MacMahon B. Dentition, oral hygiene and risk of oral cancer: a case-control study in Beijing, People's Republic of China. Cancer Causes and Control 1990; 1: 235-242.
10. Kreimer AR, Clifford GM, Boyle P and Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol. Biomarkers Prev. 2005; 14(2): 467-75.
11. Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Cogliano V. WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of alcoholic beverages. Lancet Oncol. 2007; 8(4): 292-3.
12. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283(15): 2008-12
13. Greenland S. Quantitative methods in the review of epidemiologic literature. Epidemiol Rev. 1987; 9: 1-30
14. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21(11): 1539-58.
15. van Houwelingen HC, Arends LR and Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. Stat Med. 2002; 21(4): 589-624
16. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol. 1992; 135(11): 1301-9
17. Macaskill P, Walter SD and Irwig L. A comparison of methods to detect publication bias in meta-analysis. Stat Med. 2001; 20(4): 641-54.
18. Marques L, Eluf-Neto J, Figueiredo ROA, Góis-Filho JF, Kowalski LP, de Carvalho MB, Abrahão M, Wünsch-Filho V. Oral health, hygiene practices and oral cancer. Rev Saúde Pública 2008; 43: 471-9
19. Fernandez Garrote L, Herrero R, Ortiz Reyes RM, Vaccarella S, Lence Anta K, Ferbeye L, Muñoz N, Franceschi S. Risk factors for cancer of the oral cavity and oro-pharynx in Cuba. Brit J Cancer 2001; 85: 46-54.
20. Winn DM, Blot WJ, McLaughlin JK, Austin DF, Greenberg RS, Preston-Martin S, et al. Mouthwash use and oral conditions in the risk of oral and pharyngeal cancer. Cancer Res. 1991; 51: 3044-7.
21. Winn DM, Diehl SR, Brown LM, Harty LC, Bravo-Otero E, Fraumeni Jr JF, et al. Mouthwash in the etiology of oral cancer in Puerto Rico. Cancer Causes Control 2001; 12: 419-29.

22. Divaris K, Olshan AF, Smith J, Bell ME, Weissler MC, Funkhouser WK, Bradshaw PT. Oral health and risk for head and neck squamous cell carcinoma: the Carolina Head and Neck Cancer Study. Cancer Causes Control 2010; 21: 567-75
23. Macfarlane TV, Macfarlane GJ, Oliver RJ, Benhamou S, Bouchardy C, Ahrens W, Pohlabeln H, Lagiou P, Lagiou A, Castellsague X, Agudo A, Merletti F, Richiardi L, Kjaerheim K, Slamova A, Schejbalova M, Canova C, Simonato L, Talamini R, Barzan L, Conway DI, McKinney PA, Znaor A, Lowry RJ, Thomson P, Healy CM, McCartan BE, Marron M, Hashibe M, and Brennan P. The aetiology of upper aerodigestive tract cancers among young adults in Europe: the ARCAGE study. Cancer Causes Control. 2010; 21: 2213-21.
24. La Vecchia C, Tavani A, Franceschi S, Levi F, Corrao G, Negri E. Epidemiology and prevention of oral cancer. Oral Oncol. 1997; 33: 302-12.
25. Guha N, Boffetta P, Wunsch Filho V, Eluf Neto J, Shangina O, Zaridze D, et al. Oral health and risk of squamous cell carcinoma of the head and neck and esophagus: results of two multicentric case–control studies. Am J Epidemiol. 2007; 166: 1159-73.
26. Blot WJ, Winn DM, Fraumeni Jr JF. Oral cancer and mouthwash. J Natl Cancer Inst. 1983; 70: 251-3.
27. Wynder EL, Kabat G, Rosenberg S, Levenstein M. Oral cancer and mouthwash use. J Natl Cancer Inst. 1983; 70: 255-60.
28. Young TB, Ford CN, Brandenburg JH. An epidemiologic study of oral cancer in a statewide network. Am J Otolaryngol. 1986; 7: 200-8.
29. Mashberg A, Barsa P, Grossman ML. A study of the relationship between mouthwash use and oral and pharyngeal cancer. J Am Dent Assoc. 1985; 110: 731-4.
30. Kabat GC, Hebert JR, Wynder EL. Risk factors for oral cancer in women. Cancer Res. 1989; 49: 2803-6.
31. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case–control study of human papillomavirus and oropharyngeal cancer. New Engl J Med. 2007; 356: 1944-56.
32. Weaver A, Fleming SM, Smith DB. Mouthwash and oral cancer: carcinogen or coincidence? J Oral Surg. 1979; 37: 250-3.
33. Mascarenhas AK, Allen CM and Moeschberger ML. The association between Viadent use and oral leukoplakia--results of a matched case-control study. J Publ Health Dent. 2002; 62: 158-62
34. Morse D, Katz R, Pendrys D, et al. Mouthwash use and dentures in relation to oral epithelial dysplasia. Oral Oncol. 1997; 33: 338-43.
35. Marshall JR, Graham S, Haughey BP, Shedd D, O'Shea R, Brasure J, et al. Smoking, alcohol, dentition and diet in the epidemiology of oral cancer. Eur J Cancer B Oral Oncol. 1992; 28B: 9-15.
36. Elmore JG, Horwitz RI. Oral cancer and mouthwash use: evaluation of the epidemiologic evidence. Otolaryngol Head Neck Surg. 1995; 113: 253-61.
37. Shapiro S, Castellana JV, Sprafka JM. Alcohol-containing mouthwashes and oropharyngeal cancer: a spurious association due to under-ascertainment of confounders? Am J Epidemiol. 1996; 144: 1091-5.
38. Cole P, Rodu B, Mathisen A. Alcohol-containing mouthwash and oropharyngeal cancer: a review of the epidemiology. J Am Dent Assoc. 2003; 134: 1079-87.